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Research to Determine the Accumulation of Organic Constituents and Heavy Metals from Petroleum-Impacted Sediments by Marine Detritivores of the Alaskan Outer Continental Shelf

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To the
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Administration, OCSEAP Office
Juneau, Alaska

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RESEARCH TO DETERMINE THE ACCUMULATION OF ORGANIC CONSTITUENTS AND HEAVY METALS FROM PETROLEUM-IMPACTED SEDIMENTS BY MARINE DETRITIVORES OF THE ALASKAN OUTER CONTINENTAL SHELF

by

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ANNUAL REPORT

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PREFACE

For the past 22 months, individuals in the Battelle Marine Research Laboratory at Sequim, Washington, have been studying the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted sediments. Since our study is relevant to petroleum development of the Alaskan Outer Continental" Shelf, Prudhoe Bay crude oil was used as a test oil. Our test animals were cold-water species of the Pacific Northwest, similar to those found on the Alaskan shelf. Results of our investigation will be found in three publications, which will be available in 1978. The majority of the information contained in this report has been extracted from these manuscripts which are available in pre-print form for researchers interested in-more details.

PUBLICATIONS

Anderson, J. W., G. Roesijadi and E. A. Crecelius. 1977. Bioavailability of hydrocarbons and heavy metals to marine detritivores from oil-impacted sediments. Paper presented at the OCSEAP Review-Workshop, Seattle, Washington, October, 1977.

Roesijadi, G., D. L. Woodruff, J. W. Anderson. 1978. Bioavailability of naphthalenes from marine sediments artificially contaminated with Prudhoe Bay crude oil. Environmental Pollution (in press).

Roesijadi, G., J. W. Anderson, J. W. Blaylock. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: Influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Bd. Canada (in press).

Roesijadi, G. and J. W. Anderson. 1978. Condition index and free amino acid content of *Macoma inquinata* exposed to oil-contaminated marine sediments. *In:* 1977 Symposium on Pollution and Physiology of Marine Organisms. Georgetown, S. C., ed. by Winona and F. J. Vernberg, Academic Press, New York (in press).

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ABSTRACT

During FY1977 and 1978, experiments were conducted to examine the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted marine sediments. The feasibility of using bivalve condition index and free amino acid pool as indicators of stress due to petroleum exposure was also tested. Prudhoe Bay crude was the test oil in all experiments.

When simultaneously exposed to 600 µg/g oil in sediment for 40 days in the field, detectable levels of hydrocarbons were present in two deposit-feeding species, *Phascolosoma agassizii* and *Macoma inquinata*, but not in *Protothaca staminea*, a filter-feeder. These results suggest that mode of feeding is a determinate factor in the availability of sediment-sorbed hydrocarbons to benthic animals. Tissue magnification of hydrocarbon concentrations above those in or on sediments was not observed.

Additional short-term experiments with ^{14}C-labeled specific aromatic hydrocarbons in the laboratory indicated that ingestion of contaminated sediment resulted in negligible uptake of 2-methyl naphthalene by *Macoma inquinata*. Methylnaphthalene released from sediment to seawater appeared to be the primary contributor to tissue concentrations of this compound. Uptake of ^{14}C-phenanthrene, -dimethylbenzanthracene, and -benzo(a)pyrene, however, exhibited components which could be attributed to both direct uptake from sediment and uptake from seawater. Magnification factors showed that hydrocarbons were concentrated from seawater but not from sediment. Long-term exposure indicated that uptake of ^{14}C-benzo(a)pyrene by *M. inquinata* was linear for at least six weeks. No indication of a steady-state tissue concentration was observed.

Both free amino acid content and condition index of *Macoma inquinata* were sensitive to stress, as they showed significant reductions, compared to control animals, during field exposure to oiled sediment.

Compared to sediment concentrations, nickel, copper, zinc, and manganese were elevated in *Phascolosoma agassizii*, and nickel, zinc, and selenium in *Macoma inquinata*. Other compounds were present at levels similar to or lower than those of sediment. Exposure to oil-contaminated sediment did not appear to affect trace metals content of either species. Individual variation of trace metals content in *M. inquinata* was relatively low. Coefficient of variation for all elements ranged from 5 to 20%. Use of neutron-activated natural detritus in exposures of *Macoma* provided a closer examination of low levels of four trace metals (Co, Eu, Sc and Zn). Determinations of total gamma-activity and these specific isotopes on shells and in tissues at various intervals showed that little if any of the metals were taken up from oiled and non-oiled detritus.

Recent research with a mud-ingesting polychaete, *Abarenicola pacifica*, indicates that this species takes up and retains more phenanthrene than naphthalenes. With this organism and our experimental system, it has been possible to detect behavioral modifications, ingestion (= egestion) rate reduction, and decreases in phenanthrene content of sediment after passage through the gut.

INTRODUCTION

With increasing petroleum utilization and transport, there has been a concomitant increase in the amount of petroleum hydrocarbons that enter the marine environment. Charter *et al.* (1973) estimated that the total influx of petroleum to the oceans exceeds 3×10^6 tons per year. Numerous studies have now been conducted on interactions between oil-contaminated seawater and marine organisms. Considerable information is available on the toxicity, uptake, and deputation, metabolism, and physiological effects of these compounds (Anderson *et al.*, 1974; Neff *et al.*, 1976a; Malins, 1977; Anderson, 1977). Although it is known that hydrocarbon levels are elevated in marine sediments in the vicinity of petroleum inputs such as oil spills (Blumer *et al.*, 1970; Gilfillan *et al.*, 1976), sewage effluents (Barrington and Quinn, 1973), and refinery operations (Wharfe, 1975), little is known about the effects of oil-contaminated sediments on benthic organisms. Shaw *et al.* (1976) reported increased mortalities of clams *Macoma balthica* exposed to oiled sediment, while Rossi (1977) and Anderson *et* aZ. (1977) found little or no uptake of naphthalenes from oil-contaminated mud or detritus by a polychaete. Furthermore, there is no information regarding interactions between marine organisms and trace metals from oil-impacted sediments.

Our study has been concerned with the bioavailability of petroleum hydrocarbons and trace metals from petroleum-contaminated marine sediments using diverse experimental approaches. Two species were emphasized as test organisms in the first 18 months of the study: a detritivorous clam, *Macoma inquinata*, and a sediment-ingesting sipunculid, *Phascolosoma agassizii*. A filter-feeding clam, *Protothaca staminea*, has been used in some studies to provide a comparison between detritivores and filter-feeders. We have conducted preliminary studies

with a mud-ingesting polychaete, *Abarenicola pacifica*, which provides several experimental advantages. Exposures utilized sand, mud, and detritus (from natural sources) under laboratory and field conditions. Several analytical techniques were employed to quantify hydrocarbons in animal tissues and sediment: ultraviolet and infrared spectrophotometry, gas chromatography, high-pressure liquid chromatography, and liquid scintillation spectrometry. Trace metals were analyzed by x-ray fluorescence or neutron-activation analysis.

To date, we have conducted experiments to examine the following: (1) comparison of bioavailability of petroleum hydrocarbons from sediment in benthic deposit- and filter-feeders; (2) uptake of specific aromatic compounds from sediment in short-term experiments, differentiating between the relative importance of uptake from sediment versus seawater; (3) long-term uptake of specific hydrocarbons from sediment; (4) condition index and free amino acid content of oil-exposed clams, and (5) uptake of trace metals from oil-contaminated sediment. The results are presented in this report. Prudhoe Bay crude oil was the test oil in all experiments.

INFLUENCE OF FEEDING TYPE OF BIOAVAILABILITY OF PETROLEUM HYDROCARBONS FROM SEDIMENT

Benthic organisms are represented by species which exhibit diverse feeding modes. When considering the problem of uptake of material from sediment, it is reasonable to presume that organisms which feed directly on sediment or detritus would have a greater opportunity for accumulation from sediment than species which do not. We tested this hypothesis by exposing filter-feeding, detritus-feeding, and sediment-ingesting species to oil-contaminated sediment in a field experiment, then analyzing the organisms for tissue hydrocarbon concentrations. The clams *Protothaca staminea* and *Macoma inquinata* and sipunculid *Phascolosoma agassizii* were chosen as test species representative of the respective feeding modes listed above.

Details of experimental procedures have been described in our 1"977 Annual Report. and Roesijadi *et al*, (1978a). The results are presented in Table 1.

Concentrations of total petroleum hydrocarbons (IR) in exposure sediment were 887.4 ppm initially, then declined to 443.8 and 420.6 ppm at 40 and 60 days, respectively. The decreases can probably be attributed to microbial- and photo-oxidation of hydrocarbons as well as their release to the surrounding seawater. Although our exposure concentrations were relatively high, even higher levels have been reported after actual oil spills.

Accumulation of petroleum hydrocarbons was considerably higher in the deposit-feeders, *Macoma inquinata* and *Phascolosoma agassizii*, than in the filter-feeder *Protothaca staminea* (Table 1), indicating that deposit-feeding benthic animals are more likely to take up such compounds from contaminated sediment than are filter-feeders. At the 40 day sampling interval (Table 1),

Table 1. Aliphatic and diaromatic hydrocarbons in samples[1] of *Phascolosoma agassizii*, *Macoma inquinata*, and *Protothaca staminea* exposed to oil-contaminated sediment.

Species	Treatment	Hydrocarbon Concentrations (µg/g wet weight)					
		Saturates C_{12}-C_{28}	N	MN	DMN	Total[2] Diaromatics	Total Hydrocarbons measured
P. agassizii	Control	<0.10	<0.005	<0.005	<0.01	<0.02	<0.12
M. inquinata		<0.10	<0.005	<0.005	<0.01	<0.02	<0.12
P. staminea		<0.10	<0.005	<0.005	<0.01	<0.02	<0.12
P. agassizii	40 day exposed	1.90	<0.005	0.23	0.60	0.83	2.73
P. agassizii		0.73	<0.005	0.01	0.15	0.16	0.89
M. inquinata		0.69	<0.005	0.06	0.89	0.96	1.65
P. staminea		<0.10	<0.005	<0.005	<0.10	<0.02	<0.12
P. agassizzi	60 day exposed	1.48	0.01	0.06	0.81	0.25	1.73
M. inquinata		0.54	0.02	0.27	2.39	2.68	3.22
M. inquinata		3.62	0.02	0.26	1.96	2.24	5.86
P. staminea		0.10	<0.005	0.02	0.16	0.18	0.28
M. inquinata	60 day exposed	0.35	<0.005	0.03	0.96	0.99	1.34
	7 day depurated	0.15	<0.005	0.02	0.20	0.22	0.37
P. staminea		0.03	<0.005	<0.005	<0.01	<0.02	0.03
		0.02	<0.005	<0.005	0.01	0.01	0.03

[1] Each sample consisted of 2 to 4 pooled individuals; clams were shucked prior to extraction.

[2] Total diaromatics include naphthalene (N), methylnaphthalenes (MN), and dimethylnaphthalenes (DMN).

hydrocarbon levels in *Protothaca staminea* were below our detection limits, while those in *M. inquinata* and *Phascolosoma agassizii* ranged between 1 to 3 ppm . combined aliphatics and diaromatics. Relative contributions of the two fractions were similar for both species. Aliphatics averaged 1.1 ppm, and total diaromatics averaged 0.7 ppm. The diaromatics consisted of the alkylated forms, particularly the di- and tri-methylnaphthalenes. Naphthalene was not detected. At 60 days (Table 1), hydrocarbon concentrations in *Macoma* were higher than those at 40 days, primarily due to increases in levels of di-methylnaphthalenes. The apparent increase in uptake between 40 and 60 days is difficult to explain; however, we have observed a similar phenomenon with benzo(a)pyrene uptake from sediment by *M. inquinata*. At the 60 day sampling, *Protothaca staminea* also contained a small amount of petroleum hydrocarbon, approximately 0.3 ppm combined aliphatics and total aromatics (Table 1). Transfer of exposed M. *inquinata* and *Protothaca staminea* to clean seawater for one week resulted in significant deputation of both saturate and aromatic hydrocarbons from clam tissue (Table 1).

For comparative purposes, we exposed *Protothaca staminea*, the filter-feeder to 0.02 - 0.03 ppm Prudhoe Bay crude oil dispersed in seawater for 60 days in a continuous-flow bioassay system. The results indicated that tissue hydrocarbon levels were considerably higher than those in the exposure seawater and were consistent with previous reports on the uptake of petroleum hydrocarbons from seawater by marine bivalves. Approximately 11 ppm aliphatic and total aromatic hydrocarbons were present in clam tissue, with a distribution pattern similar to that described above for animals exposed to oil-contaminated sediment.

It is evident from our study that the feeding type of benthic organisms is an important factor in the bioavailability of hydrocarbons from sediment.

Both the aliphatic and diaromatic petroleum hydrocarbons on or in marine sediments are more readily taken up by detritivores than filter-feeders. However, the extent of accumulation was relatively low compared to initial sediment hydrocarbon concentrations. Since concentrations in tissue of both *Phascolosoma agassizii* and *Macoma inquinata* increased during the 60 days of the experiment, the long-term implications for bioaccumulation cannot be adequately defined at the present time. In a study using oiled sediment similar to that reported here, concentrations in sediment of docosane, naphthalene, and phenanthrene exhibited exponential decreases with approximate half-times of 40 days. Our IR analyses also indicated a decrease of petroleum hydrocarbons with exposure time. Thus, it would appear that animals in our experiment were accumulating hydrocarbons during a period which coincided with release of these compounds from sediment.

UPTAKE OF ^{14}C-LABELED AROMATIC HYDROCARBONS BY MACOMA *INQUINATA* IN SHORT-TERM EXPERIMENTS

Our efforts consisted of short-term (1 week) experiments to survey the relative uptake of various aromatic hydrocarbons from oil-contaminated sediments. The objective was to screen several compounds in an attempt to identify those which may have greater significance with respect to bioavailability from marine sediments. We selected *Macoma inquinata* as a test species, since preliminary observations indicated that this clam is an active detritus-feeder. The test compounds were Z-methyl naphthalene, phenanthrene, chrysene, dimethylbenzanthracene, and benzo(a)pyrene.

Clams were collected from intertidal regions of Sequim Bay, Washington, and held at the Marine Research Laboratory of Battelle-Northwest, Sequim,

Washington. Holding tanks contained raw, flowing seawater of about 10°C and 30°/₀₀ and sediment obtained from the vicinity of the clams' natural habitat.

Detrital material which settles out of our flowing seawater system was collected" and filtered onto No. 42 Whatman filter paper. Fifteen grams were weighed and suspended in approximately 30 ml Prudhoe Bay crude oil dissolved together in 1 ml ethyl ether were added to the suspended detritus, mixed thoroughly by shaking, then filtered onto No. 42 Whatman filter paper. The contaminated detritus was used in exposures. Stock solutions of ^{14}C-hydrocarbons were tested for radioisotope purity by thin-layer chromatography and autoradiography. Measurements by infrared spectrophotometry (IR) indicated approximately 2,000 µg/g total hydrocarbons in the detritus.

Since oil-contaminated sediments can release hydrocarbons to the surrounding water, it was necessary to consider the possibility of uptake of solubilized, as well as sediment-bound, hydrocarbons. Therefore, some clams were placed on the bottom of exposure aquaria containing the contaminated detritus, while others were placed in a nylon-mesh (Nitex) basket suspended in the water column above the detritus. The first group fed directly on the detritus, and the latter served as a control for uptake from the water. Seven-day exposures were conducted in all-glass aquaria containing detritus and 3 ℓ of 0.45µ filtered seawater. At the end of exposure, some individuals from the bottom and suspended basket were removed for immediate extraction, while the remainder were transferred to clean seawater for a 24-h gut purging period.

Net uptake from sediment, i.e., the amount of hydrocarbon ingested and present in clam tissue at the end of the exposure period, can be calculated as follows:

Net uptake = Concentration in clams on bottom - concentration due to seawater uptake - concentration in gut contents + concentration lost from tissue during gut purging.

If uptake is primarily due to absorption of solubilized hydrocarbons, then the value for actual uptake would be essentially zero or negative.

Seawater samples were taken prior to the addition of clams and at 1, 2, 4, and 7 days. Detritus was sampled initially and at 7 days. All samples were analyzed by liquid scintillation spectrometry and corrected for quench. Additional experimental details are described in our 1977 Annual Report.

The results are summarized in Table 2. There was no measurable uptake of the diaromatic 2-methylnaphthalene from sediment. Uptake from seawater could account for the entire amount of this substance in clam tissue. Higher molecular weight compounds possessed an uptake component associated with net uptake from sediment. Comparison of net uptake from seawater indicated that both sources contributed similar amounts to the tissue burden of polyaromatic hydrocarbons. Magnification factors indicated that hydrocarbons in sediment were not as readily accumulated by clams as hydrocarbons in seawater. Sediment magnification factors were typically less than 0.1, while seawater magnification factors ranged from 3.2 to 420. Furthermore, seawater magnification factors exhibited a correlation with molecular weight of the aromatic compound, increasing with increasing size of compound. Such a correlation is undoubtedly related to the lipid vs. water solubilities of the compounds. Thus, larger molecular weight compounds which are more lipophilic would tend to have a greater affinity for animal tissues than smaller compounds. Sediment magnification factors did not exhibit such a trend.

Table 2. Uptake of ^{14}C-polyaromatic hydrocarbons from sediment by *Macoma inquinata*. Clams were exposed to sediment containing 2000 ppm crude oil spiked with 10 μC of the hydrocarbon indicated in the table.

Parameter	2-Methyl-Naphthalene	Phenanthrene	Chrysene	Dimethyl-Benzanthracene	Benzo(a)pyrene
Net Uptake From Sediment[1] (μg/g)	0	0.096	0.308	0.297	0.059
Uptake From Seawater (μg/g)	0.048	0.038	0.297	0.856	0.037
Sediment Magnification Factor[2]	0	0.056	0.029	0.039	0.057
Seawater Magnification Factor[3]	3.2	5.89	105	295	420

[1] Calculated As Indicated in Text

[2] Sediment Magnification Factor = Net Uptake/Geometric Mean Concentration In Sediment

[3] Seawater Magnification Factor = Uptake From Seawater/Geometric Mean Concentration In Seawater.

UPTAKE OF ^{14}C-AROMATIC HYDROCARBONS BY *MACOMA INQUINATA* IN A LONG-TERM EXPERIMENT

We examined long-term uptake of phenanthrene, chrysene, and benzo(a)pyrene from sediment by *Macoma inquinata*. Since short-term experiments, already described, indicated a low level of accumulation of these compounds by *M. inquinata*, it was necessary to determine if prolonged exposure would also produce similar results.

Clams were collected in the intertidal region of Sequim Bay and held in the laboratory in flowing seawater of approximately 10°C and 30°/$_{oo}$. Exposures were conducted in compartmentalized sediment trays already described. Each compartment was filled with 3 kg clean sand and placed in holding tanks with flowing seawater and a simulated diurnal tidal flux. Cement blocks held the trays at a level that prevented "high tide" from overflowing the upper edges of the sediment trays. "Low tide" completely drained seawater from the trays through fiberglass mesh bottoms. Therefore, the only water flux in the exposure trays occurred through the tray bottoms as the trays drained and filled. Twenty clams were placed in each compartment. Six exposure and one control trays were prepared.

Contaminated detritus was prepared as described for short-term experiments. At "high tide" approximately 25 g of suspended detritus was added to each compartment and allowed to settle on the surface of the sand containing clams. Clams and sediment were sampled at 3, 7, 14, 28, and 42 days of exposure. Each sampling period entailed removal of all clams and one sediment core from a compartment. Half the clams and the sediment core were extracted and analyzed immediately. The remaining clams were transferred to clean seawater for 24 h to allow purging of gut contents, then analyzed.

During the course of exposure, the detritus which had settled onto the surface of the sand penetrated into interstitial spaces as a result of the tidal fluxes. Since it was impossible to separate detritus from sand at sampling intervals after clay 3, counts for core samples were used as a measure of hydrocarbon content. For purposes of comparison, initial counts for detritus were corrected to account for the total sediment load (= detritus + sand), assuming uniform distribution of the detritus in sand. These values could then be directly compared to values for core samples.

For phenanthrene and chrysene, ^{14}C-radioactivity was also separated into parent compound and metabolize fractions using a procedure described by Roubal *et al.* (1977).

Concentrations of radioactivity in sediment are described in Figure 1. Initial concentrations were similar (- 1.0×10^4 dpm/g) for phenanthrene, chrysene, and benzo(a)pyrene and exhibited an apparent two-component exponential decrease with time. Phenanthrene, the smallest compound, decreased at a faster rate than chrysene or benzo(a)pyrene. Final sediment ^{14}C concentrations were two orders of magnitude less than initial levels with phenanthrene and approximately one order of magnitude less with chrysene and benzo(a)pyrene. In all three cases, loss rates were relatively rapid.

Behavior of ^{14}C-radioactivity in tissue of exposed clams was different for the three compounds and apparently related to relative solubilities (Figure 2). With all three compounds, exposed clams took up an initial high dose measured at either two or three days of exposure. This initial uptake was probably associated with the high levels in the initial exposure detritus on the sand surface and active filtration of this highly contaminated material. With time, however, the detritus percolated into the underlying sand substrate as described earlier. Tissue concentrations of ^{14}C-phenanthrene radioactivity steadily

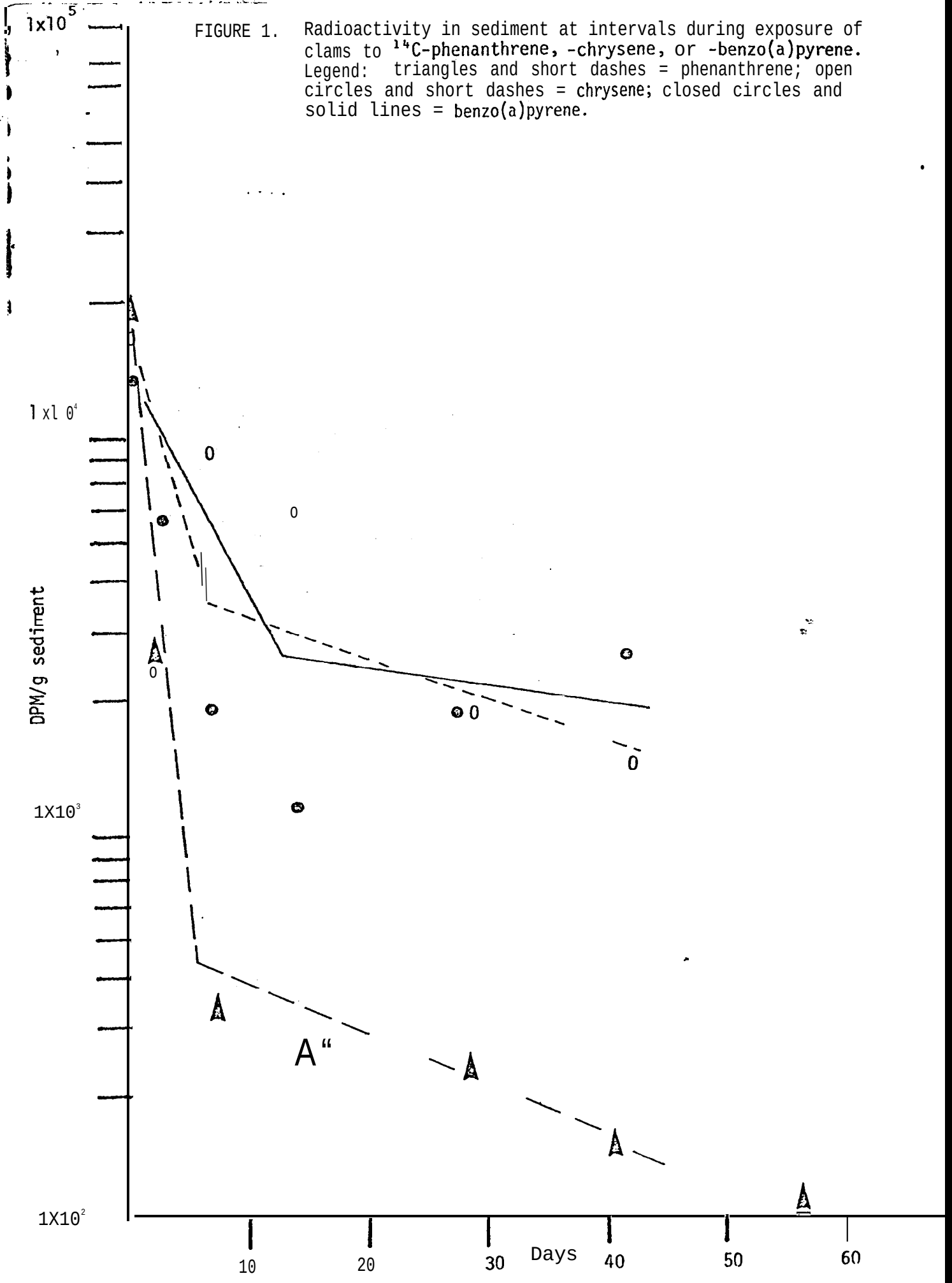
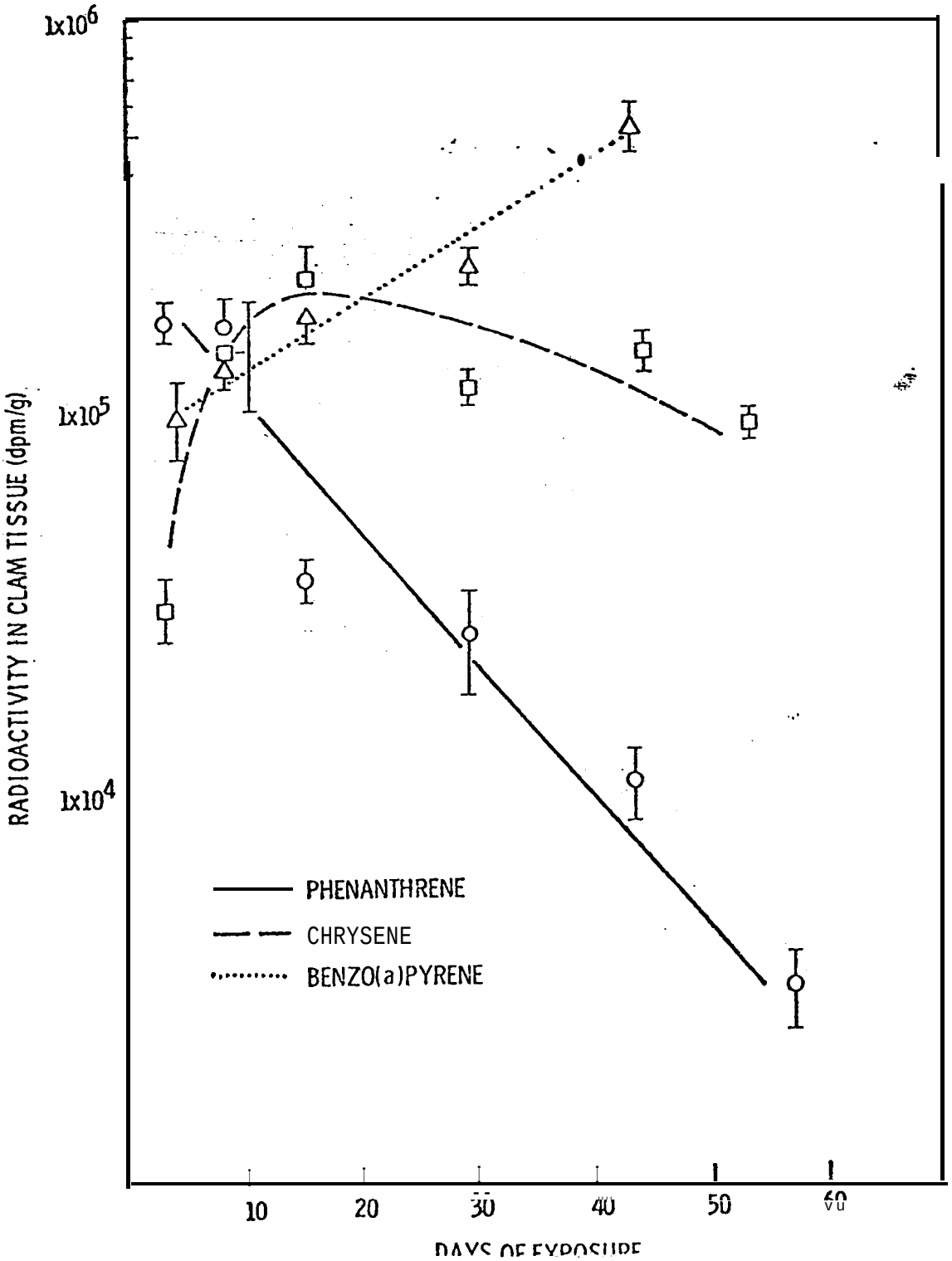


FIGURE 1. Radioactivity in sediment at intervals during exposure of clams to ^{14}C-phenanthrene, -chrysene, or -benzo(a)pyrene. Legend: triangles and short dashes = phenanthrene; open circles and short dashes = chrysene; closed circles and solid lines = benzo(a)pyrene.

Figure 2. Radioactivity in clam tissue at intervals during exposure to HC-phenanthrene, -chrysene, or -benzo(a)pyrene.

^{14}C



declined with subsequent exposure indicating an initial high uptake followed by deputation. Tissue ^{14}C-chrysene concentrations increased up to day 14, then began to decline after that time. ^{14}C-benzo(a)pyrene in tissue, however, continued to increase throughout the duration of the experiment (42 days in this case). These observed differences are probably associated with the relative solubilities of the three compounds in water and lipids. For example, net uptake as presented in Figure 2 can be described as follows:

net uptake = influx - efflux.

Therefore, net uptake is positive when influx exceeds efflux and negative when efflux exceeds influx. The kinetics of benzo(a)pyrene radioactivity uptake is clearly representative of the former case, while phenanthrene kinetics is representative of the latter. Chrysene possessed a positive net uptake during the early stages of the exposure then tissue concentrations began to decrease. Since benzo(a)pyrene was the most lipophilic compound of the three, phenanthrene the most hydrophilic, and chrysene intermediate; it appears that the relative affinities of the three compounds for clam tissue, probably the lipid pool, was associated with the behavior of these compounds in our experimental system.

Separation of ^{14}C-radioactivity for phenanthrene and chrysene into parent and metabolize fractions indicated a difference in the behavior of these compounds in both sediment and clam tissue. For example, the fraction of phenanthrene radioactivity present as parent compound in sediment decreased at a faster rate than that for chrysene (Table 3), indicating that phenanthrene was a less stable compound in our exposure system. Furthermore, the fraction of phenanthrene radioactivity in clam tissue associated with parent compound decreased from 97.1 to 44.5% of the total radioactivity over the 56 day exposure - period. For chrysene, almost all the radioactivity in clam tissue was still associated with the parent compound at the end of exposure.

Table 3. Percent of total radioactivity present as parent compound.

Compound	Time (days)	Sediment	Tissue
Phenanthrene	0	98.4	-
	2	83.5	97.1
	7	36.6	87.1
	14	35.3	80.6
	28	17.4	60.2
	42	27.2	51.2
	56	17.7	44.5
Chrysene	0	90.4	-
	2	94.7	98.4
	7	78.1	96.2
	14	63.0	95.8
	28	72.0	93.9
	43	67.4	94.7
	52	24.6	95.8

It is obvious that degradation of phenanthrene in sediment occurred at a faster rate than for chrysene, and that the relative contributions of metabolizes of phenanthrene increased with time in clam tissue. Chrysene metabolizes in tissue were negligible. Microbes and photo-chemical oxidation as well as loss to the seawater were probably responsible for the turnover of these compounds in sediment. At the present time, there is no evidence to suggest that marine bivalves possess enzymatic systems which can degrade aromatic hydrocarbons. Therefore, phenanthrene metabolizes present in clam tissue may have originated in the sediment and were subsequently taken up by clams.

DISTRIBUTION OF ^{14}C-RADIOACTIVITY IN TISSUES

The analytical chemistry associated with determining the rates of uptake, level of accumulation and fate of ^{14}C-labeled aromatic hydrocarbons derived from exposing intertidal detritivores to oil contaminated substrate requires the solution of two analytical tasks. These tasks are, A: analysis of poly-aromatic hydrocarbons in oil and, B: in tissue.

In the first task, high pressure reverse phase liquid chromatography (HPLC) employing ultraviolet and fluorescence detection systems is being used to determine the concentrations of chrysene and benzo(a)pyrene in Prudhoe Bay crude oil (PBC) which are two of three compounds used in the accumulation studies. Isolation of a polyaromatic enriched fraction from the crude oil utilizes aspects of a procedure described by Pancirov *et al.* (1975). The concentration of phenanthrene in PBC, the third compound to be used in these studies, has been determined by capillary gas chromatography. Since these compounds are present in PBC oil, these data are needed to relate levels of

radioactivity to amounts of each of these compounds (specific activity) to which the organisms are exposed as a function of time.

Reverse-phase HPLC. has previously been used in a method to characterize impurities associated with benzo(a)pyrene degradation (Clarke, 1976), and we have recently used this technique to determine the radio-purity of all three substrates used in these studies. Phenanthrene and chrysene undergo no significant chemical degradation under prolonged storage, however, benzo(a)pyrene showed the presence of about 4% impurities and, therefore, will have to be verified by silica gel chromatography prior to each experiment to assure that no degradative chemical artifacts are introduced to invalidate radioactivity analysis.

In the second task, a method is being developed to monitor the uptake and fate of ^{14}C-phenanthrene, chrysene and benzo(a)pyrene in tissue of intertidal detritivores. The method will allow us to account for the formation of metabolites or conjugates which are of interest in future studies. Conventional tissue digestion techniques such as that described by Warner (1976) cannot be used in the method because of potential degradation of metabolizes or conjugates. Therefore, initial preparation includes homogenization of tissue samples in an organic solvent. Removal of high molecular weight components (biogenic) from the tissue extracts is necessary to reduce separation interferences and minimize quenching effects. Gel permeation chromatography incorporates a modified version of a method described by Keuhl *et* aZ. (1978}. Samples isolated from this system containing initial substrate and any associated metabolizes or conjugates are further fractionated by reverse-phase HPLC, and the amount of radioactivity associated with parent hydrocarbon substrate and metabolizes are determined using liquid scintillation counting.

UPTAKE OF HYDROCARBONS FROM MUD BY *ABARENICOLA*

Work has begun on *Abarenicola pacifica*, a polychaete which presents several advantages for the assessment of bioavailability of petroleum hydrocarbons or other pollutants from muddy sediment. *A. pacifica* occupies an L-shaped burrow constructed in mud. The anterior 2/3 of the animal normally lies in the deep horizontal portion of the burrow (the gallery or head shaft) about 10 cm below the surface, while the posterior 1/3 lies in the vertical tail shaft. Undulating movements of the body, especially the tail, bring in currents of water for respiration and, to some extent, *for* feeding. Micro- and meio-organisms suspended in the respiratory current are filtered on the walls of the end of the head shaft and are ingested together with the surrounding sediment. The resulting excavation, as well as the hydraulic abrasion of the respiratory current, cause a subsidence of the sediment lying over the animal's anterior end. Thus, material lying on the surface of the sediment can, over a period of several days, be drawn down to the level of the head shaft and be ingested. At intervals the posterior end of the animal is raised to the upper end of the tail shaft, and feces are deposited around the opening in a characteristic pattern.

As a result of this mode of life, materials adsorbed onto sediments may be presented to *A. pacifica* via several routes. Materials that remain attached to sediment may be ingested directly. Materials that are given off into the water column may be taken up through the respiratory current or be incorporated into suspended food organisms which will then be eaten. Furthermore, sediment' at all levels above the head shaft is subject to ingestion. Since the fecal material from individual worms can be easily collected, it is possible to analyze changes in pollutant material resulting from passage through the digestive tract.

Preliminary investigations have been conducted with a small number of these organisms to determine whether they respond to hydrocarbon contamination , by avoiding it. For this purpose, four plastic boxes with Nitex mesh bottoms were divided into two compartments by a teflon sheet. Clean sediment from the worms' habitat was placed in one half of each tray. The other half was filled with sediment into which 1000 ppm PBC oil had been stirred with a motor driven impeller. The teflon sheets were removed, one worm was introduced into each box at the dividing line between clean and oiled sediment, and the boxes were placed in tanks with flowing seawater. The location of the worms after feeding could be determined by the location of the fecal casts surrounding the tail shafts. Initially, two of the four worms moved into the oiled mud and two into clean mud. After ten days, one worm moved from the oiled to the clean mud, and one had moved in the opposite direction. Three weeks later however, all the animals had moved into the unoiled halves, and they remained there until the termination of the experiment (35 days).

The uptake and release of naphthalenes by *Abarenicola* from contaminated sediments were studied by mixing PBC with substrate at a high (H) concentration of 1000 ppm and at a low (L) concentration of 100 ppm. Worms were exposed to the sediment, either directly (D) by being placed in trays entirely filled with the contaminated sediment, or indirectly (I) where they were in a 2 cm deep layer of unoiled mud overlain by a 5 cm layer of oiled mud. The four combinations of conditions are designated as HD, HI, LD, and LI. The oil-mud mixtures were placed in mesh-bottomed trays in running seawater for four hours and flushed twice by changes in water level before the worms were placed in them. The trays were then placed in clean running seawater.

At intervals of several days, a tray was removed and the worms it contained were washed free of surface contamination with a stream of distilled water.

The intestinal tract of each animal was removed, slit opened and freed of its contents by flushing with distilled water. The cleaned guts, body wall * and part of the coelomic fluid of each animal were frozen together at -80°C in teflon-lined, hexane-washed, centrifuge tubes and saved for later analysis. Tissue samples were taken at 1, 4, 7, 14, and 22 days of exposure. Worms exposed to HI conditions for eight days were moved to clean sediment for deputation of one and eight days. Frozen tissue samples were thawed and analyzed for naphthalene, methylnaphthalenes, and dimethylnaphthalenes contents by UV spectrophotometry (Neff and Anderson, 1975). Samples of sediment and fecal casts were taken for IR analysis of total hydrocarbons.

The results of this set of experiments are summarized in Table 4. As might be expected, the average concentration of tissue naphthalenes was highest under HD conditions, less in HI, still less in LD, and lowest in LI. As much as 3 ppm total naphthalenes was present in the HI *Abarenicola* in four days. Under those conditions in which a time course of accumulation and deputation was followed, a plateau appeared to be reached within a few days of exposure and loss of naphthalenes following transfer to clean sediment was rapid, leading to a 90% decrease in concentration within eight days. The concentration of naphthalenes in the sediment could not be measured directly due to its high organic content., but calculation based on an unpublished analysis of PBC indicated that the highest tissue concentration reached was of the same order of magnitude as the sediment concentration but somewhat lower. Since the preliminary flushing of oiled sediment undoubtedly removed an unknown proportion of the naphthalenes, it is not possible to say whether a tissue magnification effect exists.

IR spectrophotometry indicated that the total hydrocarbon content of the H and L sediments, when corrected for the endogenous hydrocarbons of the native

Table 4.. Uptake of naphthalenes (N + MNs + DMNs) by *Abarenicola pacifica* exposed to oiled mud. The UV technique (Neff and Anderson, 1975) showed that control tissue produced a background fluorescence of 0-0.2 ppm naphthalenes. All values listed are ppm total naphthalenes and means are shown for each interval.

Condition	Exposure (days)					Deputation (days) after 8 days exposure	
	1	4	7	14	22	1	8
High Concentration (471 ppm)							
In oiled mud[1] (Direct)					2.15 4.23 3.19		
Below oiled mud[2] (Indirect)	1.46 2.16 1.81 1.81	1.44 2.57 3.46 2.49	1.09 4.20 2.65	2.6 1.99 1.60 2.06		0.99 0.90 0.43 0.39 0.22 0.76 0.32	
Low Concentration (52 ppm) In oiled mud (Direct)	0.58 0.63 1.03 0.75	0.99 0.60 0.80	0.93 0.93				
Below oiled mud (Indirect]	0.36 0.53 0.34 0.41	0.66 0.54 0.77 0.56	0.73 0.37 0.55				

[1] The polychaetes were completely surrounded by oil contaminated mud.

[2] The animals were initially in a 2 cm deep layer of clean mud overlain by a 5 cm layer of oiled sediment.

substrate (68 ppm), differed by a factor of ten (471 and 52 ppm, respectively). The average absolute value of the added oil, measured by IR methods, was one half of the calculated value measured by volume. Few fecal casts were produced by worms in the D sediment. Their hydrocarbon content (HD = 538 ppm; LD = 145 ppm) closely matched the total average content of the sediment surrounding the worms. Fecal casts collected from I worms had lower average contents (HI = 171 ppm; LI = 93 ppm).

Uptake of another aromatic compound was studied under conditions that allowed direct comparisons between sediment, tissue, and fecal concentrations. In this experiment sediment containing 1000 ppm PBC, to which ^{14}C-labeled phenanthrene had been added at 85% of its endogenous concentration, was placed in ten U-shaped tygon tubes, 40 cm long. The tubes were suspended in flowing seawater and one worm was placed in each. Feces were collected daily from plastic trays surrounding the ends of the tubes. After twelve days the sediment and surviving worms were removed. The radioactivity of sediment, worms, and feces were measured by liquid scintillation, following extraction of phenanthrene by a modification of the method of Warner (1976). Contaminating material was rinsed from the worms' exteriors and their gut lumens with ethanol, assuring that only the phenanthrene incorporated into the tissues was measured. Interstitial water was extracted from the sediment by centrifuging for 20 minutes at 29,000 g, followed by passage through a .45μ Millipore filter. Control animals were placed in similar tubes containing unoiled sediment, such that survival and fecal cast production could be compared.

Several differences between the controls and experimental are noteworthy. During the 12 days, one control and five exposed animals died. The exposed animals appeared to be in more distress since their tails were seen to protrude from their burrows 20 times versus 3 times for the controls (Table 5). This

Table 5. Effects of oiled mud on the feeding and behavior of *A. pacifica*. Mud contained a calculated concentration of 1000 ppm PBC oil of " which 0.56 ppm was phenanthrene.

Response	Controls	Exposed
Survival	90%	50%
Tails projecting outside of tubes (no. of events)	.3	20
Fecal casts produced	31	17
Mean weight of - casts/g live tissue	1 .25g	0. 92g
Mean fecal production	0.5g/day/g tissue	0.2g/day/g tissue

behavior, which is clearly non-adaptive in the field, is never seen under natural conditions. On the other hand, two of the control worms and none of the exposed left their tubes.

The rate of feeding was lower in the exposed group, which produced 17 fecal casts in 11 days, among seven animals which either survived or produced at least one cast (Table 5}. Nine control animals in the same categories produced 31 casts. It is of interest that only three of the experimental group produced casts during the first week of exposure, and two of these failed to survive, indicating that cessation of feeding in the early stages of oil contamination may be a protective response.

There is a clear effect of oil on the rate of turnover of sediment by *Abarenicola pacifica* populations, as the control group produced 0.5 g feces/day/g live weight of animal, and the exposed only 0,2 g. The difference is partly due to the lower rate of cast production by the exposed and partly from the lower average size of the casts, .92 g/g live weight versus 1.25 g for the controls (Table 5).

The average concentration of phenanthrene within the tissues of the exposed worms was higher than that in the surrounding sediment, though of the same order of magnitude, with the highest concentration-of any found in the body of a dead worm (Table 6). The tissue concentration was not affected by the rate of feeding, as the same level, corresponding to a total (endogenous plus labeled) level of about 80 ppb, was found in an animal that had produced no fecal casts as in one that had passed more than its own body weight in sediment through its intestinal tract.

It may be conjectured that high concentrations of petroleum hydrocarbons can enter *A. pacifica* by direct contact with water and surrounding sediment as

Table 6. Uptake of ^{14}C-Phenanthrene from oiled mud by A. *pacifica*. 100 µC of * ^{14}C-Phenanthrene (1.57 mg) was added to 3.2 g of PBC (1.86 mg phenanthrene) and this was mixed with 3.2 kg of mud.

Compartment	Mean ^{14}C-activity (dpm/g)	Concentration (ppm) mean ± S.D.	Number of Samples
Mud	38$400	0.56 ± .0.14	9
Whole worm (survivors)	53,200	0.80 ± 0.19	5
Whole worm (deaths)	84,000	1.25	2
Body wall	38,500	0.57	1
Gut	115,000	1.72	1
Feces	9,790	0.16 ± 0.09	12
Interstitial water	80	0.0013	7

well as through ingested material, though the level of phenanthrene in interstitial water was two to three orders of magnitude less than that in the sediment (Table 6). Further experimentation is being planned to determine the relative importance of these routes.

There is some evidence that the digestive tract does play a significant role in the response of *A. pacifica to* petroleum since the gut of one animal, which was analyzed separately, had three times as high a concentration as the remaining tissue. Furthermore, the phenanthrene content of the feces in nearly all cases was substantially lower than that of the ingested sediment, indicating that it had been metabolized into a more soluble form during its transit through the gut.

UPTAKE OF TRACE ELEMENTS FROM OIL-CONTAMINATED SEDIMENT

In last years Annual Report, data were presented on the concentrations of trace elements in Prudhoe Bay crude oil, test sediments and detritus and tissues exposed to clean or oiled substrate. The crude oil was analyzed by neutron-activation analysis, and all other samples were characterized by x-ray fluorescence. Both *Phascolosoma agassizii* and *Macoma inquinata* were used in these studies, and between 14 and 22 different trace elements were determined in the experimental substrates and tissues. Trace metals of biological significance which were determined include V, Cr, Ni, Cu, Zn, Pb, Sr, As and Hg. To determine variability between individual *Macoma*, replicate analyses (3-10 samples) were conducted and values for two standard errors were generally 10% of the mean or less (Table 7). The exposure of *Macoma* in laboratory or field experiments to clean and oiled substrate did not produce concentrations of any trace elements in the tissues which were above normal variations. The data generated in FY1977 for both *Macoma* and

Table 7. Analysis of trace elements in *Macoma inquinata* by x-ray fluorescence. Estimation of sample variability.

Element	Sample size[1]	Concentration (µg/g) $\bar{x} \pm 2$ S.E.
P	10	4,651 ± 686
S	10	15,374 ± 591
Cl	10	53,859 ± 3,695
K	10	13,504 ± 245
Ca	10	2,003 ± 140
Ti	10	23.7 ± 9.5
V	3	3.58 ± 0.45
Cr	5	3.92 ± 0.60
Mn	10	9.136 ± 1.043
Fe	10	315.2 ± 32.3
Co	4	2.497 ± 0.442
Ni	10	3.282 ± 0.391
Cu	10	8.108 ± 0.374
Zn	10	195.2 ± 12.5
Ga	0	n.d.[2]
Hg	10	n.d
Se	10	3.177 ± 0.188
Pb	3	0.815 ± 0.680
As	10	10.319 ± 0.368
Br	10	262.5 ± 17.8
Rb	10	n.d.
Sr	10	29.59 ± 2.46

Phascolosoma suggested that within the limits of the analytical approaches utilized, these organisms were not exhibiting uptake of trace metals from oiled substrate.

Since the possibility existed that the x-ray fluorescence techniques may not be detecting small changes in the tissue content of certain heavy metals, we suggested the use of radio-labeled detritus and oil which would be produced from neutron-activation of these substances. By generating gamma-emitting isotopes from the metals contained in the oil and associated with the detritus, very small amounts of isotopes transferred from these substances to the detritivores could be measured. In the fall of 1977 (FY1978), samples of oil and detritus were subjected to neutron-activation, and the products were measured for isotope content and activity. Because the concentration of metals in the oil was so low (Table 8) and the specific metals present did not lend themselves to use in this experimentation, the activated oil was not utilized.

The detritus, however, did possess at least four gamma-emitting isotopes, which exhibited activities and half-lives suitable for use in experimentation. In January of 1978, a preliminary experiment was conducted to evaluate the uptake of isotonically-labeled heavy metals by the clam, *Macoma inquinata*. Activated natural detritus was mixed with fresh cold detritus (1:10), and the mixture was "aged" in seawater at 10"C for four days. The final product was then filtered on #42 Whatman paper, and divided into two halves. The oil-impacted portion received a calculated 2000 ppm of PBC contamination by the methods described earlier under hydrocarbon exposure. The non-oiled portion received only one ml of ether used as a carrier in the oiled sample. These two samples of activated detritus were placed on the bottom of two separate

Table 8. Trace element concentrations in Prudhoe Bay Crude oil Samples represent oil from two different barrels and were analyzed by neutron activation analysis.

Element	Concentration (µg/g)	
	Sample 1	Sample 2
Na	<0.06	0.097
Mg	<30	<33
Al	<0.5	<0.5
cl	<1	0.95
K	<4	<1.4
Sc	<0.001	<0.001
v	20.9	18.0
Cr	<0.21	<0.15
Mn	<0.04	<0.02
Fe	<1.6	<1.7
Co	0.018	0.017
Cu	<5	<3
Zn	0.31	0.31
As	<0.03	<0.01
Se	--	<0.3
Br	5.73	2.75
Rb	<0.06	<0.08
In	<0. 005	<0.003
Sb	<0.002	<0.002
Cs	<0.002	<0.001
Ba	<23	<8
La	<0.01	<0.01
Sm	<0.002	<0.001
Eu	<0.001	<0.001
Tb	<0.007	<0.006
Ta	<0.04	--
Hg	<cl. 03	<0.03
Th	<0.008	<0.006

5-liter aquaria and low aeration was supplied. Ten marked clams were placed on the substrate of each tank, and then a basket containing an additional five clams was suspended in the water column above the other animals. The exposure continued for one week and there was an additional deputation period of two days. During the exposure, water and clam samples were counted at 1, 3, and 7 days, and animals were also counted after deputation. It was possible to utilize a small number of animals since they could be counted alive and placed back in the aquarium. The same groups of five individuals were counted together, and the configuration within the counting chamber was kept constant. After the one week exposure and final counting at Sequim for total gamma activity, five of the ten animals on the bottom of each aquarium were transferred to clean water with clean detritus for two days deputation. The remaining five in each group were removed from the shell, and both tissue and shell were sent to Richland for detailed analyses. The same procedure was used on the 2-day depurated groups and the two groups of five suspended above the detritus. The determinations of total gamma activity and specific isotope content of the various groups and samples are shown in Tables 9 and 10.

During the one week exposure, the oiled detritus decreased in total hydrocarbon concentration from 1755 ppm to 1138 ppm. The gamma activity associated with the water above the detritus (both oiled and non-oiled) was primarily in solution and was of significant magnitude, except on day 3 (Table 9). Counts generally present in the 200 ml samples were about twice as high as those found in the clam tissues after seven days of exposure to detritus (200). Clams suspended above the substrate, where activity could only be obtained from the water and very fine suspended particles, exhibited rather consistent counts between 34 and 79. The shells of clams living on the bottom of both aquaria (oiled and unoiled detritus) possessed a total of 35 counts/g (per 40 min.).

Table 9. Uptake of total gamma-labeled trace metals, from detritus by *Macoma*. Values are counts per 40 minutes per gram (tissue) or per 200 ml (seawater).

Type of Sample	Sample Interval (days) Exposure				Deputation (after 7 days exposure)
	0	1	3	7	2
Seawater (200 ml)					
Filtered (0.5μ)	370	432	23	440	
Unfiltered	473				
Filter	263				
Macoma					
On Detritus					
With oil		109	141	200	45
Without oil		230	59	172	52
Above detritus					
With oil		79	54	6a	27
Without oil		68	34	55	23
Shell only				35	28

Two days of deputation in clean water and detritus reduced all counts, including those clams on and above detritus, with and without oil and shells, , to a range of 23 to 52 (Table 9). Since the shell alone gave a count of 28, it is apparent that uptake by clam tissue was extremely small if present at all.

Samples taken on the seventh day of exposure and after two days of deputation were analyzed for content of specific radioisotopes (Table 10). It is clear that the detritus contained sufficiently high amounts of these four isotopes to provide the organisms with an opportunity to exhibit uptake. ^{60}Co was found in the water at higher counts than the other metals, but these only represented 1% of the detritus activity and the Zn in water represented about 4% of detrital activity. There are no apparent differences in the activity of clams between the oiled and non-oiled groups, but the sub-groups living above the detritus both exhibited lower activity. Deputation for two days reduced the levels of activity to those of the clams living above the substrate, which is approximately equal to that associated with the shell of those living on the detritus.

It is interesting to note that when the Zn counts in the tissues are converted by use of the Zn specific activity, the amount of Zn accumulated by *Macoma* represents only about 0.1% of the total Zn found in freshly collected " animals. These findings make two facts apparent. First, no other means of analysis would ever detect uptake of Zn at this very low level; and secondly, a short deputation reduces tissue levels to approximately the same activity associated with shell material. These findings agree with our 1977 report, which indicates that trace metals are probably not available from sediments, *even* in the presence of oil.

We feel that the question of trace metals availability from oil-impacted sediment is fairly well answered. However, we plan one fins"l experiment using

Table 10. Uptake of Specific Radio-Labeled Trace Metals from Detritus by *Macoma*. Values are counts per 1000 minutes per gram detritus or tissue and per 200 ml seawater.

	Isotopes			
	^{152}Eu	^{60}Co	^{46}Sc	^{65}Zn
Detritus (after 7 days)				
With oil	25,984	13,456	14,528	1,216
Without oil	17,038	17,038	9,656	913
Seawater (200 ml)				
Filtered on Day 7				
With oil	0	131	1	41
Without oil	14	173	7	28
Filter				
With oil	6	1	9	7
Without oil	8	6	5	<1
7-day *Macoma*				
With oil				
On detritus	101	70	5	5 16
Above detritus	10	13	4	7
Without oil				
On detritus	81	65	48	13
Above detritus	7	11	4	8
2-day Deputation (on detritus)				
With oil	15	19	8	7
Without oil	23	26	10	13
Shell only				
7-day Exposed on detritus				
With oil	39	24	22	7
Without oil	50	28	23	8
7-day Exposed above detritus				
With oil	16	9	8	5
Without oil	13	9	7	4
2-day Depurated (on detritus)				
With oil	37	22	20	7
Without oil	10	10	10	4

detritus containing a larger number of isotopes and higher specific activity. The results of this last study, if in agreement with earlier findings, should provide ample information for evaluating trace metal transfer from oiled substrates.

CONDITION INDEX AND FREE AMINO ACIDS OF *MACOMA INQUINATA* EXPOSED TO OIL-CONTAMINATED SEDIMENTS

Our study has shown that both condition index and certain free amino acids in *Macoma inquinata* were significantly altered by exposure to oil-contaminated sediments (Tables 11 and 12). Adequate sample size was an important factor in demonstrating statistically significant reductions in condition (Table 11). The reduction in condition index in exposed clams, although small (-10%}, provided evidence of a deterioration in nutritional state. A decrease in bivalve condition index is an indication that affected clams may have been in a state of negative-energy balance; in other words, metabolized energy exceeded energy consumed as food. Utilization of endogenous storage products such as tissue protein, lipid, and carbohydrates may have been necessary to provide the balance of the energy for metabolism under such conditions (Gabbott, 1976). Condition index of oysters and mussels have been closely correlated with tissue glycogen content (Walne, 1970; Gabbott and Stephenson, 1974; Gabbott and Bayne, 1973).

Using the criteria proposed by de Wilde (1975) for *Macoma balthica*, a value of >10 represents good condition, ~8 moderate, and <6 poor. If these criteria are applicable to *M. inquinata*, the clams in this study possessed mean values for condition index which ranged from good to moderate. Condition in bivalves is known to undergo seasonal variations which are reflective of reproductive and nutritional state (Maine, 1970; Trevallion, 1971; de Wilde, 1975). Periodic sampling of clams from our collection site during the course

Table 11. Condition index of *Macoma inquinata* exposed to oil-contaminated sediment in the second experiment. Exposure was conducted in the field only. (From Roesijadi and Anderson, 1978).

Treatment	Sample size (n)	Condition index
Control	91	8.92 ± 0.18 (S.E.)***
Exposed	50	7.46 ± 0.28

*** Significant at $p<0.001$; Student[s *t* test

of this study indicated that condition index of our experimental clams, especially those used in field exposures, were out of phase with the natural population. In general, clams in this study possessed lower condition index than those collected freshly at the times o-F experiment termination (13.79 ± 1.63 and 17.01 ± 0.78 for experiments 1 and 2, respectively). Thus, experimental manipulation also was a factor which influenced condition index of clams in this study. The effect of oil-exposure on condition was, therefore, either additive or synergistic with general experimental conditions.

Since arginine, lysine and threonine are considered to be essential amino acids (Mahler and Cordes, 1971), decreases in these substances in oil-exposed clams were also suggestive of alterations in nutritive state. Increased utilization of these amino acids, possibly in protein sythesis, by oil-exposed clams or a decrease in their ingestion with food may have accounted for our observations. The large decrease in glycine content in our oil-exposed clams was consistent with previous studies which examined free amino acid levels in marine animals subjected to pollutant or natural stresses (Jeffries, 1972; Roesijadi *et aZ.,* 1976; Bayne *et al.*, 1976). As a consequence of the decrease in glycine, the taurine:glycine ratio was elevated in oil-exposed clams (Table 12). The actual values of 0.54 ± 0.06 (S.E.) for control clams and 0.89 ± 0.19 (S.E.) for exposed clams in this study were not directly comparable to those reported by Jeffries (1972) or Bayne *et aZ.* (1976) since taurine levels in *Macoma inquinata* were much lower than those in the bivalves *Mercenaria mercenaria* and *Mytilus edulis* used in the other studies. Although taurine:glycine ratios may prove useful in identifying bivalves which have experienced stressful environmental conditions> it is evident that the cause of the change in the ratios is due primarily to alterations in glycine content. This pattern has been consistent in the studies conducted to date. Examination of glycine metabolism would certainly be useful in understanding this apparent stress response.

Table 12. Free amino acid content of *Macoma inquinata* exposed to oil-contaminated sediment in the second experiment. Conducted in the field only. (From Roesijadi and Anderson, 1978).

Amino acid	Concentration (μ moles/g) Control	Exposed	
Alanine	22.53 ± 1.82 (S.E.)	16.80 ± 2.02 (S. E.)	*
Arginine	6.89 ± 0.40	4.56 ± 0.73	
Aspartate	1.44 ± 0.17	0.90 ± 0.27	
Glutamate	2.72 ± ().22	2.21 ± 0.19	
Glycine	70.25 ± 4.51	43.56 ± 4.99	**
Histidine	0.28 ± 0.03	0.22 ± 0.04	
Isoleucine	0.37 ± 0.02	0.32 ± 0.04	
Leucine	0.63 ± 0.05	0.48 ± 0.05	
Lysine	0.59 ± 0.04	0.41 ± 0.04	**
Methionine	0.20 ± 0.03	0.13 ± 0.02	
Phenylalanine	0.22 ± 0.02	0.19 ± 0.02	
Proline	0.57 ± 0.04	0.74 ± 0.25	
Serine	4.09 ± 0.41	3.24 ± 0.39	
Threonine	1.16 ± 0005	0.87 ± 0.07	**
Tyrosine	0.37 ± 0.03	0.33 ± 0.03	
Valine	0.54 ± 0.04	0.44 ± 0.16	
Taurine	37.06 ± 1.94	35.08 ± 2.39	
Total	150.57 ± 8.15	110.48 ± 8.24	**
Taurine:Glycine	0.54 ± 0.04	0.89 ± 0.12	*

* Significant at $p<0.02$, Student's *t* test

** Significant at $p<0.01$, Student's *t* test

CONCLUSIONS

There appears to be a tissue accumulation pattern associated with the molecular weight of the petroleum hydrocarbon, which is probably dependent on relative partitioning coefficients. From other studies it seems that tissue retention times, while animals are in clean water, increase with the size of the hydrocarbon, and the number of side chains (alkylation). In other words, deputation time increases in the approximate order of: naphthalene, > methylnaphthalenes > dimethylnaphthalenes > phenanthrene > methyl- and dimethyl phenanthrenes > chrysene > benzo(a)pyrene. No single study with one organism and one type of system has been conducted to produce these data, but the basic pattern appears to exist.

We must assume that tissue retention time for a given compound is independent of the route by which an organism received the contamination. There is some evidence that hydrocarbons entering via food may be retained longer, but there is no reason to believe that there are differences associated with water, interstitial water and sediment routes. Therefore, once the hydrocarbons reach the tissue the rates of release (by various means) should be dependent upon relative solubilities in tissue lipids vs. tissue water (and subsequently surrounding water). The rapid uptake and short-term retention of naphthalenes we have observed in these and earlier studies are probably explained by uptake from water. In a relatively short time, sediments give up (release) naphthalenes to the interstitial water and this compartment eventually exchanges with the overlying water column. If we assume that this is also the sequence of events for higher molecular weight compounds, then the majority of our findings may be explained by differences in the rates of equilibration between sediment-sorbed and 'water-born hydrocarbons. This line of thought leads to the conclusion

that all uptake observed was via the water (including interstitial water), and ingestion of' contaminated particles does not result in significant tissue contamination. This hypothesis would appear to be strengthened by the data on trace metals. These substances were not accumulated by the two test species, even though sediments contaminated with oil were ingested. Either the metals were bound tightly to the particles or there was merely an exchange taking place which resulted in no net uptake.

Our most recent research with the burrowing polychaete, *Abarenicola*, produced results which indicate that uptake from ingested mud does occur. Those animals which fed on oiled substrate in the early stages of exposure did not survive, and the one digestive tract which was analyzed exhibited higher accumulation than the body wall. Since the concentration of ^{14}C-activity in interstitial water was about two orders of magnitude less than the mud, it is difficult to explain the mortality and uptake merely on a basis of the interstitial water. It will be easier to assess the significance of the various routes of uptake when our analyses include a separation of parent hydrocarbons and metabolizes. It is possible that a portion of the activity observed in the gut was metabolic products, since polychaetes possess higher levels of detoxification enzymes in their gut than other tissues.

During both laboratory and field exposures of *Macoma* to oiled sediment, the condition index of the organisms decreased to a greater extent than occurred in clean substrate. When the sample size was large enough to overcome individual variability, both condition index and free amino acid content of *Macoma* was shown to be affected by oil contamination. Experimental manipulation alone reduced conditon index below that of freshly collected animals, which indicates that a better knowledge of site and seasonal variability must be gained and experimental design should be improved. These parameters, which reflect the

energy balance of the species, have been shown to be valid and sensitive measurements for the study of pollutant effects.

The results of various aspects of our research under NOAA/BLM funding through the OCSEAP program have been submitted and accepted for publication by three different publishers (Roesijadi, Woodruff and Anderson, 1978; Roesijadi, Anderson and Blaylock, 1978; Roesijadi and Anderson, 1978). The full references for these manuscripts are listed in the literature cited section and copies of the papers are on file at the OCSEAP offices of Juneau, Alaska and Boulder, Colorado.

The application of information obtained in these studies would appear to be relatively straightforward. It is important to understand the interactions between oil, sediments and benthic organisms, once petroleum has reached the substrate. Present evidence indicates that hydrocarbons bound to sediment particles are not directly available to deposit/detritus-feeding organisms. Leaching from sediment would appear to be controlled by the water volubility of the specific compound, physical-chemical factors in the environment, and microbial activity. The physical energy of the environment would control release rates, and the dilution volume available when release occurs will control the" extent of uptake by benthic organisms. Even when tissue uptake is relatively low the condition of organisms may be reduced, probably from decreased feeding on contaminated substrate. The recovery of a single generation in oiled substrate or the recovery of a specific benthic habitat would also be controlled by the physical-chemical and microbiological factors in that environment. Not all of those assumptions are sufficiently validated by scientific investigations, but if future studies are designed to fill the information gaps, we will be in a position to predict the fate and effects of petroleum hydrocarbons in marine sediments.

RECOMMENDATIONS AND FUTURE DIRECTION

There is a need to continue sediment studies to test the hypotheses proposed above regarding the factors controlling sediment recovery after oiling. While generating these data, we can also determine the effects of sediment-sorbed hydrocarbons on infaunal species and the rates and routes of hydrocarbon uptake. Field experiments should be designed to answer a number of questions simultaneously, thus saving time and money. With careful planning, coordination and separate funding, a large field experiment in the Alaskan environment could be conducted, using the combined expertise of many OCSEAP investigators to describe the physical, chemical, microbiological and biological parameters associated with the presence of oil on sediments. Such field experiments could either be centered around an application of oil on the interdial zone or a spill in the nearshore environment.

Variability between types of marine sediments and species of benthic organisms are such that additional data are required to determine the extent of these differences and the controlling factors. We intend to look more closely at fine (mud) sediments and to utilize a deposit feeding polychaete in these studies. It is likely that hydrocarbons will be retained longer in this system, and that we will be able to separate hydrocarbon inputs via sediment from those in the interstitial water. Condition index and free amino acids appear to be good indicators of effects on bivalves, but only the latter can be used on soft body animals and it may not be useful. While toxicity from water exposure may be linked to tissue accumulation, effects of oiled substrate may be primarily related to reduced feeding on contaminated detritus and/or sediment. Since highly contaminated substrates are often non-toxic to benthic species in the short-term, we may need to determine the levels of sediment contamination that

interfere with normal energy intake over the long-term. While trace metals were not accumulated in our experiments, we will utilize neutron-activated detritus once more to study exchange rates for metals and to look more closely for uptake.

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